PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY To:					MANS			
					PCT PCT			
				ŀ		TCI YON		
					WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY			
					(PCT Rule 43bis.1)			
					Date of mailing (day/month/year)	See form PCT/ISA/210		
١ ٠٠		gent's file referen	ce		FOR FURTHER			
	137P					See paragraph 2 below		
l		plication No.	266	International filing date ('day/month/year)	Priority date (day/month/year) 24.03.2004		
		2005/003				24.03.2004		
l			(IPC) or both	national classification an	d IPC			
C12	2Q1/	58						
Applica	ani		· · · · · · ·					
I		OMICS AG			•			
					•			
					-,			
1.		pinion contains if	adications rela	ting to the following items	::			
l	\bowtie	Box No. I	Basis of the	opinion				
[\boxtimes	Box No. II	Priority		egard to novelty, inventive step and industrial applicability s.1(a)(i) with regard to novelty, inventive step or industrial ons supporting such statement			
	Ш	Box No. III	Non-establis	shment of opinion with re				
		Box No. IV	Lack of unit	y of invention				
	\boxtimes	Box No. V						
	\boxtimes	Box No. VI	Certain doct	uments cited	·			
		Box No. VII	Certain defe	cts in the international ap	plication			
		Box No. VIII	Certain obse	ervations on the internation	onal application			
2.	רסווק	THER ACTION						
	If a c Intern than t	lemand for intere ational Prelimina his one to be the	y Examining. IPEA and the	Authority ("IPEA") excep	t that this does not app I the International Bur-	If be considered to be a written opinion of the ply where the applicant chooses an Authority other eau under Rule $66.1bis(b)$ that written opinions of		
	If this writte	opinion is, as pr n reply together,	ovided above, where approp	considered to be a writte	n opinion of the IPEA before the expiration	A the applicant is invited to submit to the IPEA a of 3 months from the date of mailing of Form expires later.		
	For fu	rther options, see	Form PCT/IS.	A/220.				
3.	For fu	rther details, see i	notes to Form	PCT/ISA/220.				
<u> </u>								
Name a	and maili	ng address of the	ISA/EP		Authorized officer			
		•				•		
						•		
Facsim	ilc No.	<u> </u>			Telephone No.	•		

Воз	No. I	Basis of this opinion	
1.		egard to the language, this opi nless otherwise indicated unde	nion has been established on the basis of the international application in the language in which it was r this item.
	[]	his opinion has been established	ed on the basis of a translation from the original language into the following language
			, which is the language of a translation furnished for the purposes of international search (under
	F	tule 12.3 and 23.1(b)).	
2.		egard to any nucleotide and on, this opinion has been estab	for amino acid sequence disclosed in the international application and necessary to the claimed lished on the basis of:
	a. t	ype of material	
	D	a sequence listing	
		table(s) related to the sequ	nence listing
	b. f	ormat of material	
	D	in written format	
	Ē	in computer readable form	n
	c. ti	me of filing/furnishing	
	Γ	contained in the internation	onal application as filed.
		~	crnational application in computer readable form.
	L N		this Authority for the purposes of search.
	Ľ	Turning subsequently to	and realisting for the purposes of section.
3.	f	arnished, the required statemen	ore than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or ats that the information in the subsequent or additional copies is identical to that in the application as application as filed, as appropriate, were furnished.
4.	Additio	onal comments:	
		•	
		•	
,			

Box No. II Priority	
l. The following document has not yet been furnished:	
copy of the earlier application whose priority has been claimed (Rule 43bis.1 and 66.7(a)).	
translation of the earlier application whose priority has been claimed (Rule 43bis.1 and 66.7(b)).	
Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been establish the assumption that the relevant date in the claimed priority date.	
2. This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found in (Rules 43bis.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to relevant date.	nvalid be the
3. Additional observations, if necessary:	
The validity of the priority claim has not been	
considered because the International Searching Authority	
does not have in its possession a copy of the earlier	
application whose priority has been claimed or, where	
required, a translation of that earlier application. This	
opinion has nevertheless been established on the	
assumption that the relevant date (Rules 43bis1 and 64.1)	
is the claimed priority date.	
·	
<u> </u>	
	•

Box No. V		Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement					
1.	Statement	· • • • • • • • • • • • • • • • • • • •					
	Novelt	y (N)	Claims	1-22	YES		
			Claims		NO		
	Inventive step (IS) . Industrial applicability (IA)		Claims	1-22	YES		
			Claims		NO		
			Claims	1-22	YES		
			Claims		NO		
2.	1)	and explanations: Reference	is m	ade to the following documents:			
		D1: COTT	RELL .	S E ET AL: "A real-time PCR assay			
		for	DNA-m	ethylation using methylation-			
		spec	ific	blockers" NUCLEIC ACIDS RESEARCH,			
		OXFO	RD UN	IVERSITY PRESS, SURREY, GB, vol. 32,			
		no.	1, 13	January 2004 (2004-01-13), page			
		e10.					
		D2: WO 0	2/070	735 A (AKZO NOBEL N.V; DEIMAN,			
		BIRG	IT, A	LBERTA, LOUISA, MARIA; FRANTZEN,			
		INGE) 12	September 2002 (2002-09-12).			
		D3: WO 9	7/477	62 A (SARNOFF CORPORATION) 18			
		Dece	mber	1997 (1997-12-18).			
	2)	Document	D1 is	considered the prior art closest to			
		the subje	ct ma	tter of claim 1. It discloses a			
		method of	anal	yzing DNA methylation, comprising			
		bisulfite	conv	ersion of the DNA, with any non-			
		methylate	d cyt	osine bases being converted to			
		uracil wh	ile 5	-methylcytosine bases remain			
		unchanged	. Sub	sequent use of blocker			
		oligonucl	eotid	es results in an increase in PCR			
		amplifica	tion	specificity.			

International application No.
PCT/EP2005/003366

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The subject matter of the application differs from that of D1 in that the method includes a transcription-based amplification step. The subject matter of the application is therefore novel over D1 (PCT Article 33(2)).

with respect to D1, the problem addressed by the present invention can be considered that of providing an improved DNA methylation analysis. The application solves this problem by using a transcription-based amplification step in the claimed method. Documents D2 and D3 have previously disclosed methods of amplifying DNA by using a transcription-based amplification. However, non of the documents D1 to D3 includes any indication of combining the method of D1 with the transcription-based amplification according to D2 and/or D3. For this reason, the claimed subject matter of protection appears to involve an inventive step (PCT Article 33(3)).

		10)	•	
	Application No. Patent No.	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim (day/month/year)
	. •			
	•		•	
		•		
			•	
		•	•	
	•			
Non-	written disclosures (Rule 43bis.1 and 70.9)			
			D	ate of written disclosure
	Kind of non-written disclosure	Date of non-written d	isclosure referri	ate of written disclosure ing to non-written disclosure (day/month/year)
	Kind of non-written disclosure	Date of non-written d (day/month/yea	isclosure referri	
- •	Kind of non-written disclosure		isclosure referri	ing to non-written disclosure
	Kind of non-written disclosure		isclosure referri	ing to non-written disclosure
	Kind of non-written disclosure		isclosure referri	ing to non-written disclosure
	Kind of non-written disclosure		isclosure referri	ing to non-written disclosure
•	Kind of non-written disclosure		isclosure referri	ing to non-written disclosure
•	Kind of non-written disclosure		isclosure referri	ing to non-written disclosure
•	Kind of non-written disclosure		isclosure referri	ing to non-written disclosure
•	Kind of non-written disclosure		isclosure referri	ing to non-written disclosure
•	Kind of non-written disclosure		isclosure referri	ing to non-written disclosure
•	Kind of non-written disclosure		isclosure referri	ing to non-written disclosure
•	Kind of non-written disclosure		isclosure referri	ing to non-written disclosure
•			isclosure referri	ing to non-written disclosure
ee f	Kind of non-written disclosure		isclosure referri	ing to non-written disclosure
ee f		(day/month/yec	isclosure referri	ing to non-written disclosure

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTH	ORITY		ANS.
То:			PCT PCT
			RITTEN OPINION OF THE IONAL SEARCHING AUTHORITY
			(PCT Rule 43bis.1)
		Date of mailing (day/month/year)	See form PCT/ISA/210
Applicant's or agent's file reference		FOR FURTHER	ACTION
P1437PC00		<u></u>	See paragraph 2 below
International application No. PCT/EP2005/003366	International filing date 24.03.2005	(day/month/year)	Priority date (day/month/year) 24.03.2004
International Patent Classification (IPC) or C12Q1/68	ooth national classification an	d IPC	
Applicant EPIGENOMICS AG			
Box No. II Priority Box No. III Non-est Box No. IV Lack of Box No. V Reasone applicat Box No. VI Certain Box No. VII Certain Box No. VIII Certain Certain Priority Box No. VIII Certain Box No. VIII Certain Priority If a demand for international printernational Preliminary Examin than this one to be the IPEA and this International Searching Auth	ablishment of opinion with re- unity of invention d statement under Rule 43bis idity: citations and explanatio documents cited defects in the international ap- observations on the internation reliminary examination is re- ing Authority ("IPITA") exce- the chosen IPEA has notifie ority will not be so considered ove, considered to be a writt propriate, with amendments tion of 22 months from the p IVIS A/220.	gard to novelty, inven- th (a)(i) with regard to ons supporting such sta uplication onal application ande, this opinion w pt that this does not ap d the International Bu d. en opinion of the IPE before the expiratio	ill be considered to be a written opinion of the oply where the applicant chooses an Authority other reau under Rule 66.1 bis(b) that written opinions of A, the applicant is invited to submit to the IPEA and of 3 months from the date of mailing of Form
Name and mailing address of the ISA/EP		Authorized officer	
		Talankara Na	
Facsimile No.		Telephone No.	

Вох	No. I	· I	Basis of this o	pinion						
1.				, this opinion ha ited under this it		on the bas	s of the internal	tional appli	cation in the	language in which it was
		This opi	nion has been		c basis of a transla					
				۰, ر	which is the langua	age of a tra	slation furnish	ed for the pr	rposes of in	ternational search (under
		Rule 12.	3 and 23.1(b))			•				
2.				ide and/or ami een established o		e disclosed	in the internat	tional appli	cation and	necessary to the claimed
	a.	type of n	naterial	-						
		∑ as	equence listing	· }						
		tab	le(s) related to	the sequence lis	sting					
	b.	format o	f material							
		M in	written format		•			•		
		in in	computer read	able form						
	c.		iling/furnishin				J			
	•		-	nternational app	lication as filed					
		\equiv			al application in c	omputer ro	dable form			
			_							
		i rur	nisnea subseq	uently to this Au	thority for the pur	poses or se	ircn.			
3.		furnished	l, the required	statements that	one version or c the information in ion as filed, as ap	the subseq	uent or addition	and/or table aal copies is	e(s) relating identical to	thereto has been filed or that in the application as
				•						
4.	Addi	itional con	nments:		•					
·										
			. •							
						•				-
						-			•	
				•				•		
										-
										•
l										
		4								
		,		•						
l										

Box No. II Priority
The following document has not yet been furnished:
copy of the earlier application whose priority has been claimed (Rule 43bis.1 and 66.7(a)).
translation of the earlier application whose priority has been claimed (Rule 43bis.1 and 66.7(b)).
Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date in the claimed priority date.
2. This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43bis.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:
The validity of the priority claim has not been
considered because the International Searching Authority
does not have in its possession a copy of the earlier
application whose priority has been claimed or, where
required, a translation of that earlier application. This
opinion has nevertheless been established on the
assumption that the relevant date (Rules 43bis1 and 64.1)
is the claimed priority date.
is the statmed priorie, date.
·

1. Statement Novelty (N) Claims Claims Inventive step (IS) Claims Claims 1-22 Claims Industrial applicability (IA) Claims 1-22 Cla	YES NO
Inventive step (IS) Claims Claims Industrial applicability (IA) Claims 1-22 Claims 1-22 Claims 1-22 Claims 1-22 Claims D1: COTTRELL S E ET AL: "A real-time PCR assay	NO
Inventive step (IS) Claims Claims Industrial applicability (IA) Claims Claims 1-22 Claims 1-22 Claims 1 - 22 Claims 1) Reference is made to the following documents: D1: COTTRELL S E ET AL: "A real-time PCR assay	
Industrial applicability (IA) Claims 1-22 Claims 1) Reference is made to the following documents: D1: COTTRELL S E ET AL: "A real-time PCR assay	
Industrial applicability (IA) Claims 1-22 Claims 2. Citations and explanations: 1) Reference is made to the following documents: D1: COTTRELL S E ET AL: "A real-time PCR assay	YES
2. Citations and explanations: 1) Reference is made to the following documents: D1: COTTRELL S E ET AL: "A real-time PCR assay	NO
2. Citations and explanations: 1) Reference is made to the following documents: D1: COTTRELL S E ET AL: "A real-time PCR assay	YES
1) Reference is made to the following documents: D1: COTTRELL S E ET AL: "A real-time PCR assay	NO
1) Reference is made to the following documents: D1: COTTRELL S E ET AL: "A real-time PCR assay	
D1: COTTRELL S E ET AL: "A real-time PCR assay	
for DNA-methylation using methylation-	
specific blockers" NUCLEIC ACIDS RESEARCH,	
OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 32,	
no. 1, 13 January 2004 (2004-01-13), page	
e10.	
D2: WO 02/070735 A (AKZO NOBEL N.V; DEIMAN,	
BIRGIT, ALBERTA, LOUISA, MARIA; FRANTZEN,	
INGE) 12 September 2002 (2002-09-12).	
D3: WO 97/47762 A (SARNOFF CORPORATION) 18	
December 1997 (1997-12-18).	
2) Document D1 is considered the prior art closest to	
the subject matter of claim 1. It discloses a	
method of analyzing DNA methylation, comprising	
bisulfite conversion of the DNA, with any non-	
methylated cytosine bases being converted to	
uracil while 5-methylcytosine bases remain	
unchanged. Subsequent use of blocker	
oligonucleotides results in an increase in PCR	
amplification specificity.	

International application No. PCT/EP2005/003366

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The subject matter of the application differs from that of D1 in that the method includes a transcription-based amplification step. The subject matter of the application is therefore novel over D1 (PCT Article 33(2)).

With respect to D1, the problem addressed by the present invention can be considered that of providing an improved DNA methylation analysis. The application solves this problem by using a transcription-based amplification step in the claimed method. Documents D2 and D3 have previously disclosed methods of amplifying DNA by using a transcription-based amplification. However, non of the documents D1 to D3 includes any indication of combining the method of D1 with the transcription-based amplification according to D2 and/or D3. For this reason, the claimed subject matter of protection appears to involve an inventive step (PCT Article 33(3)).

Box	No. VI Certain docum	ents cited			
1.	Certain published documents ((Rule 43bis.1 and 70.	10)	-	
	Application Patent No		Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
		`	1-12		
		,			
					•
	•			•	
			•		
*					
			•		
			: 		
2.	Non-written disclosures (Rule	43bis.1 and 70.9)			•
	Kind of non-writte	n disclosure	Date of non-written d	lisclosure referrir	ate of written disclosure ng to non-written disclosure (day/month/year)
				·	
	•	•		÷	
					•
		٠,	•		
_	- 010		•		
See	e form 210	•			
		•			
	•	* .			